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Review

The fragile X syndrome

Angela V. Flannery *, Mark C. Hirst, Samantha J.L. Knight, Rachael J. Ritchie, Kay E. Davies

Molecular Genetics Group, Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford, OX3 9DU, UK

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Contents

1. Introduction

Mental retardation is a common disorder, affecting 2.5% of the population and ranging in severity from mild to profound. Although most often found in association with congenital or chromosomal abnormalities (e.g., Down's syndrome) or consequential to disorders of metabolism (e.g., phenylketonuria), a large number of cases are of unknown cause. It has long been recognised that males consistently outnumber females in surveys of mentally retarded individuals and linkage studies have identified many loci associated with mental retardation on the X chromosome. The most common of these X-linked disorders, accounting for 50% of cases, is the fragile X syndrome.

The identification of the molecular basis of this disorder has clarified some of the previously puzzling genetic and clinical features and has also revealed a new class of mutations responsible for inherited diseases in man.

2. Clinical and cytogenetic features

The fragile X, or Martin-Bell, syndrome was reported in 1943 [1] in the first pedigree with mental retardation linked

* Corresponding author. Present address: Genome Group, Zeneca Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, SK10 4TG, UK. Fax: +44 625 513441.

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to the X-chromosome. It has since been found to be the most common form of inherited mental retardation, with an estimated frequency of between 0.3 and 1 per 1000 live births in males and 0.2–0.6 per 1000 in females [2–4] with a worldwide distribution. The disease is inherited as an X-linked semidominant condition where 50–55% of carrier females manifest the disease and 20% of males are phenotypically normal carriers.

The fragile X syndrome was originally distinguished from the many other forms of X-linked mental retardation by the identification of a microscopic gap or constriction, termed a fragile site, in the long arm of the X chromosome at Xq27.3 in affected individuals [5–7]. The cytogenetic expression of the fragile site can be induced by culturing cells in conditions of folate deficiency, excess thymidine or in the presence of chemicals which disrupt folate metabolism (see Fig. 1). Many fragile sites have been described in human chromosomes but only two of these, the site associated with fragile X syndrome and a site located 0.6 Mb distal to it, have so far been associated with disease. Prior to the ascertainment of the molecular basis of the disease, routine diagnosis entailed time-consuming and costly cytogenetic analysis of patients and family members.

Fragile X males have mental retardation varying between moderate and profound. This is often accompanied by a typical facial dysmorphology consisting of a long face with prominent forehead, pronounced jaw and large ears. Macroorchidism can develop at puberty, suggesting the involvement of an endocrine dysfunction. This is the classical fragile X syndrome [8]. However, the clinical expression can often be extremely variable. Some patients show behavioural abnormalities such as hyperactivity, poor social contact or autism [9] and there may also be connective tissue involvement presenting as joint laxity or heart ventricular defects. Female carriers are usually less severely affected than males [10].

The syndrome shows unusual genetic characteristics in that the pattern of inheritance does not follow the classical Mendelian pattern for X-linked genes. Normal males can transmit the fragile X mutation to their daughters. These males, who are themselves clinically and cytogenetically normal, have been termed 'normal transmitting males' (NTMs). In addition, there is incomplete penetrance of the phenotype in different generations of families which segregate for the disorder. The sisters of NTMs are almost never affected and neither are their daughters. However, the sisters of affected males show a 35% penetrance for the

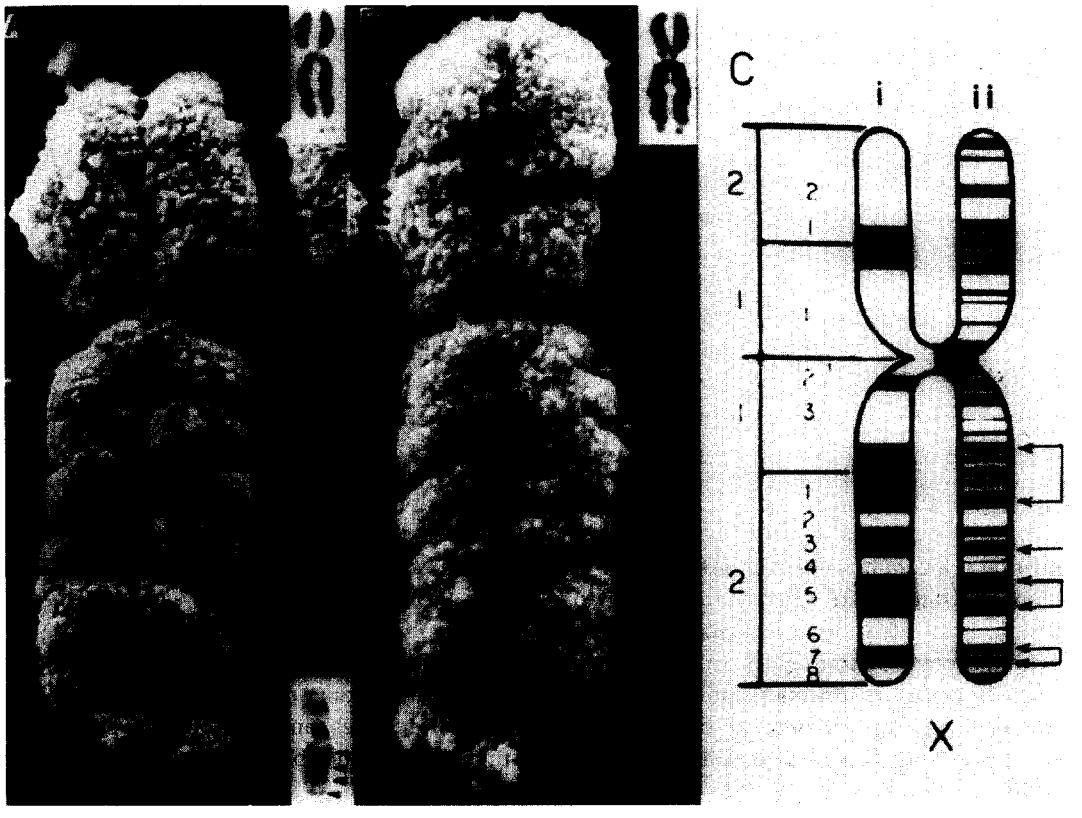

Fig. 1. Two G banded fragile X chromosomes from different metaphases viewed by Scanning Electron Microscopy (SEM). The upper inserts show non-banded chromosomes from the same individual viewed by light microscopy (LM) and demonstrating the consistency of the gross morphology. (A) The fragile site appears as an isochromatid gap in both the LM and SEM pictures. (B) The fragile site appears as a chromatid gap on the right hand chromatid, with the fragment almost completely detached. (C) A diagrammatic representation of the X chromosome showing the G positive bands (i) subdivided into a series of sub-bands (ii) indicated as arrows, also shown on the SEM pictures. The numbering system used as indicated at the left hand side (i) places the fragile site at the distal end of Xq27. Photograph reproduced from [108] courtesy of the publishers.

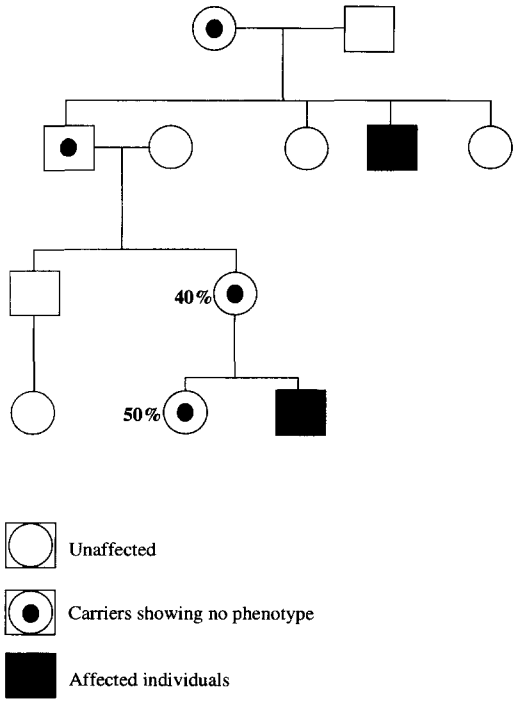

Fig. 2. Typical pedigree of a family showing inheritance of the fragile X syndrome. As the abnormal X chromosome is passed from generation to generation, the risk of mental impairment (shown as a percentage) to the offspring inheriting this X chromosome increases.

disease. The daughters of NTMs have a high probability (40%) that offspring who inherit the abnormal X chromosome will be clinically affected and the risk increases in the next generation to 50%. A typical fragile X pedigree is shown in Fig. 2. In this family, the carrier female in the first generation passes the mutation to her son, who is unaffected. The daughter of this NTM must inherit his X chromosome and she has two children, an affected boy and a carrier daughter. Her daughter has an even higher risk of having affected children. Thus the likelihood of developing mental retardation is dependent upon the position of the individual in the pedigree. This observation, which was originally known as the 'Sherman paradox' [11,12] is an example of the phenomenon called genetic anticipation. It has since been explained by the discovery that the gene associated with the disease contains a polymorphic trinucleotide CGG repeat which expands and becomes highly methylated in affected individuals, causing the gene to be silenced.

3. Isolation of the gene associated with fragile X syndrome

In common with many genetic diseases where no obvious protein candidate is available, the gene responsible for fragile X syndrome was localised by positional cloning [13]. In the case of fragile X syndrome, this process was facilitated because the fragile site localised the disease at Xq27.3.

Three approaches led to the cloning of the gene. The first involved the use of hybrid cell lines containing small portions of the X chromosome which included the fragile site region from which random DNA markers were isolated [14–16]. The second involved the production of a library containing the microdissected region of the fragile site [17]. The third approach was the construction of somatic cell hybrids containing the X chromosome from a fragile X patient on a hamster background, the hypothesis being that the human chromosomes were likely to break at the fragile site. The hybrid cells were cultured to induce fragile X expression and selection for genes on either side of the fragile site led to the isolation of several new somatic cell hybrids which contained translocations between the human and hamster material. Such events were postulated to preferentially occur in the region of the chromosomal fragility [18]. The markers generated from these studies were used to analyse genomic DNA separated by pulse field gel electrophoresis (PFGE) and a physical map of the region was constructed. The use of rare cutting enzymes, sensitive to methylation, led to the discovery that a cluster of CpG residues were specifically hypermethylated in fragile X patients [19,20]. Such clusters of methylation-sensitive sites, usually termed CpG islands, are normally unmethylated and are known to be associated with genes [21].

Yeast artificial chromosomes (YACs) spanning the methylated CpG island were shown by fluorescence in situ hybridisation to span the fragile site [22–24]. Sequencing of cosmids derived from the YACs revealed a sequence lying less than 300 bp distal to the CpG island. This sequence consists of a repeated motif of three nucleotides (CGG) which becomes abnormally amplified in individuals affected with fragile X syndrome. The expansion of this trinucleotide repeat was also suggested as the cause of the instability of the fragile X chromosomes [25,26]. At the same time, the cosmids were used to isolate an expressed sequence from fetal brain which was called FMR1 (fragile X mental retardation-1) [27]. The trinucleotide repeat was found to be present in the 5′ untranslated region of the cDNA sequence.

4. The nature of the trinucleotide repeat

The number of CGG repeats in the normal X chromosome varies between 6 and 53 copies with 29 copies occurring most frequently. Within this normal range, the alleles are stably inherited [24,28]. Allele sizes of between 54 and 200 copies are termed premutation sized alleles, since they generally belong to individuals who carry the disease gene but show no phenotypic effects. Full mutation alleles always contain more than 200 repeats. Fig. 3 shows a diagrammatic representation of the expansion of the

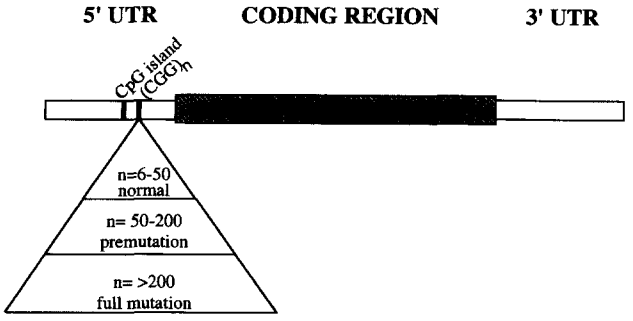

Fig. 3. Schematic representation of the FMR1 gene, showing the relative positions of the CpG island and the trinucleotide CGG repeat, which is unstable and expands to give rise to the fragile X phenotype.

repeat within the FMR1 gene. The premutation alleles are unstable and this instability increases with the size of repeat. Normal transmitting males and their daughters often show premutations with small expansions. The risk of expansion to the full mutation is dependent on the size of the premutation allele. If the repeat number is small (50–70 copies) then the risk is low; if the repeat number is high (> 90 copies) the risk is almost 100%. The risk of expansion is also dependent upon the purity of the repeat. Sequencing has shown that in normal individuals the CGG repeat can be interrupted by single base differences leading to imperfect repeats [29] and it appears that these do not show the same degree of instability as the pure repeats.

The premutation alleles change in size at female (and more rarely in male) meiotic transmission. In most instances the allele increases in size, although in rare cases reduction has also been observed [25,26]. Since affected males rarely reproduce, a very high mutation rate (1/3,000) has been suggested to maintain the frequency of the disease [30]. However, direct testing has not revealed any new mutations. A model has been proposed to account for the molecular observations [31] which postulates four alleles ranging in size from one containing a normal stable insert, two progressively larger and more unstable inserts and finally one with a large insert which gives rise to the fragile X phenotype. Evidence in support of this model has been obtained using microsatellite repeats closely linked to FMR1. These show linkage disequilibrium with the disorder indicating that a small number of founder chromosomes are responsible for approximately 55% of the observed fragile X-linked haplotypes in the Caucasian population [32–37]. Many of the chromosomes associated with these haplotypes have a CGG repeat length of approximately $n = 29$ or higher, which is close to the unstable length. These alleles may already have a slightly increased instability [25,27]. Over generations such alleles could gain more repeats which would increase their instability. The recurrence of low frequency events arising on a specific chromosome background and taking many generations to develop into a full mutation may account for both the high frequency of the disease and the finding of linkage disequilibrium.

Transmission through females is essential to generate an expansion size which will manifest the disorder. However changes in size of the CGG array can occur during both meiosis and mitosis, the latter giving rise to somatic mosaicism in fragile X individuals [25,26,28]. The presence of these two forms of instability together with X-in-

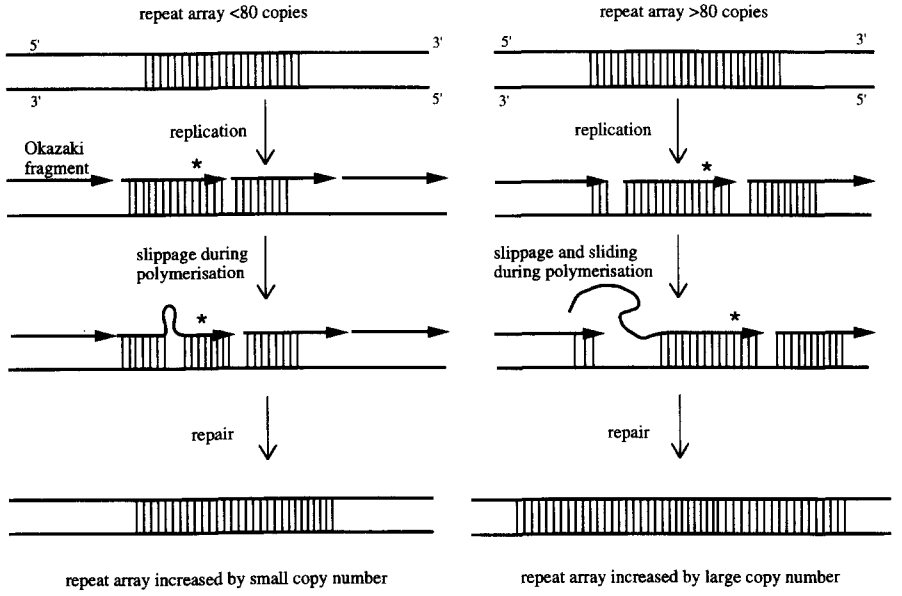

Fig. 4. Model for polymerase slippage as a method for the generation of expansions in tandem repeats. (Figure adapted from [38].) (Left) Initial copy number < 80 has a probability that only 1 single stranded break will occur during replication, consistent with the approximate length of an Okazaki fragment (*). Slippage of the elongated strand during polymerisation can result in the addition or deletion of a few copies of the repeat. (Right) Initial copy number > 80 has a probability that 2 or more single stranded breaks will occur during replication. The strand between these breaks is not anchored and is therefore free to slide during polymerisation, enabling the addition of many more copies than were present in the original sequence.

activation in females may explain why the phenotypic expression of the disease is variable.

The exact mechanism involved in the generation of expansions is not clear. The involvement of factors acting in either cis or trans have been postulated but there is little evidence thus far. One possible mechanism for expansion is based on the misalignment of replicating DNA with subsequent polymerase slippage. Fig. 4 shows a representation of the model proposed by Richards and Sutherland [38]. The mechanism requires the presence of two single strand breaks occurring within the repeat during replication. The longer the repeat, the more likely two such breaks will occur together within the sequence. In this configuration the strand of DNA between the breaks (an Okasaki fragment) is able to slip or slide during polymerisation. Such events have been observed in vitro [39]. The repair of the products of these events would be expected to lead to the introduction of many more copies of the repeat than were present in the original sequence. This could account for the generation of persistently longer alleles over many cell cycles.

An alternative mechanism, implicated in the generation of germline mutations in human minisatellites [40,41] has also been proposed to account for trinucleotide expansions. Complex gene conversion events are thought to play a role in this type mutation and such events are known to be involved in trinucleotide repeat reversions [42]. The minisatellite mutation results in a gain in copy number being polarised at one end of the repeat.

Analysis of the internal structure of the FMR1 trinucleotide repeat has shown that it is in fact a composite array with sets of CGG trinucleotides interrupted by regularly spaced AGG trinucleotides giving a defined structure to the array [29,43]. The variable length in the population is due to variable numbers of such interrupted blocks averaging nine CGG triplets. Thus a typical array would be $(CGG)_9(AGG)(CGG)_9(AGG)(CGG)_9$. Around 3–5% of the population, however, carry uninterrupted arrays with greater than 20 CGG triplets either alone or at the 3′ end of the array. These arrays occur more frequently on chromosomes with a high risk flanking haplotype, suggesting that they may be precursor arrays from which fragile X chromosomes have arisen by further slippage events. Furthermore, a comparison of the length of these arrays suggests that they have arisen by the loss of the interspersed AGG triplet, thus the reported linkage disequilibrium might be due to such a founder event.

The unstable nature of the amplification event is further complicated since it is not clear when the expansion occurs. It is known that the transition from premutation to full mutation occurs exclusively in the maternally-transmitted allele. However, it is possible that the transition occurs in the somatic cells of the developing embryo, rather than in the germline cells of the mother. Evidence suggests that fragile X affected fetuses are mosaic with respect to the trinucleotide expansion and that following the establishment of mosaicism the repeat becomes stably transmitted in somatic tissue [44,45]. These observations could result from multiple independent transitions in many different embryonic cell lineages [45]. Such a hypothesis depends upon the transition from premutation to full mutation occurring in the early zygote and could result from an imprint on the maternally derived X chromosome. Mosaic individuals with both pre and full mutation alleles would result from transition in some but not all lineages. This also implies that the transition is not present in the germline, since the mothers must contribute a premutation allele. The sperm cells of affected males show only premutation sized alleles, suggesting either that the transition does not occur in the germline, and is only present in mitotic cells [46] or that there is selection against the full mutation due to a requirement of FMR1 expression in these cells or their precursors. Direct analysis of oocytes isolated from premutation carriers is required to resolve this question.

The CGG repeat is localised in the 5′ untranslated region of FMR1 close to a CpG island which is normally unmethylated on the active X chromosome and methylated on the inactive X chromosome [25,47]. Mutant FMR1 alleles are always hypermethylated over a region which includes the 5′ CpG island and the CGG repeat [19,23,25,47,48]. In lymphoblasts, leucocytes and fibroblast of fragile X males, the FMR1 gene appears to be transcriptionally inactive [49], the loss of expression correlating with the methylation status of the CpG island. Recent work has defined the FMR1 promoter which lies within this region and has shown that it is completely inhibited by methylation in vitro [50]. Methylation of the region down-regulates FMR1 expression resulting in the disease.

It is uncertain whether abnormal methylation occurs in response to the expansion of the CGG repeat or whether methylation is responsible for the expansion. Normal transmitting males do not show methylation. Prenatal diagnosis using DNA from chorionic villi taken at the 10th week of gestation has shown the full mutation in fetal DNA both with and without methylation [35,51,52]. This is earlier than the methylation caused by lionisation, which is not yet complete by 10 weeks gestation [53]. Taken together, the data suggest that methylation occurs after the expansion.

Several lines of evidence strongly suggest that the expanded CGG repeat gives rise to the chromosomal disruption presenting as a fragile site. First, the fragile site is only seen in patients with expansions of greater than 200 repeats. A positive correlation between the mean insert size and the level of cytogenetic expression has been demonstrated [54,55]. In addition, fluorescence in situ hybridisation places the repeat in physical proximity with the fragile site [56]. Furthermore, cytogenetic expression of the fragile site does not occur in individuals whose fragile X phenotype is due to deletions or point mutations in the FMR1 gene [57–60]. Finally, the identification of another

fragile site on the X chromosome (*FRAXE*) localised 600 kb distal to FMR1 with the same underlying molecular basis (the expansion of a CGG repeat) [61] also argues in favour of the CGG repeat being responsible for the chromosomal disruption; none of the diseases caused by expansion of CAG repeats are associated with fragile sites. Delayed replication of the FMR1 gene in affected individuals has been observed, possibly due to the unusual configuration of DNA containing high GC content [62]. This phenomenon may be associated with the chromosomal fragility.

5. The FMR1 gene

Since the original discovery of the FMR1 cDNA [27], evidence has mounted that FMR1 is the primary gene involved in the fragile X phenotype. This includes the silencing of FMR1 transcription in most fragile X patients due to methylation [46], the apparent absence of other genes lying close to FMR1 [23] and the very significant finding of fragile X patients with deletions or a point mutation in the FMR1 gene [57–60,63].

The FMR1 gene is evolutionarily conserved, at least to the level of vertebrates [27]. The 4.36 kb mRNA encodes a protein of a predicted size approximately 70 kDa (631 amino acids). Alternative splicing events give rise to up to 12 distinct mRNA products, most of which differ at the 3′ end [64,65]. This may allow functional diversity, although none of the alternative isoforms has been found so far to have tissue specificity. The structure of the human gene has been determined [66] and consists of 17 exons spanning 38 kb of DNA. The intron-exon boundaries have been analysed and splice donors and acceptors at the 3′ end of the gene show less adherence to consensus, correlating with the observed alternative splicing.

6. A possible role for FMR1

No significant similarity has been found between FMR1 or its inferred amino acid sequence and any known cDNAs or proteins in the Genbank and SwissProt databases. However, analysis of predicted amino acid motifs has revealed sequences in FMR1 characteristic of RNA-binding proteins [67,68].

Amino acids 286–321 and 347–382, lying close together in the central portion of the protein, are internal repeats which have strong similarity to the KH domain which has been found to occur in hnRNP K proteins found in the hnRNP complexes involved in the biogenesis of mRNA [69]. The K protein binds strongly to sequences rich in cytidine and is the major oligo (rC/dC)-binding protein in vertebrate cells. The KH domain has also been identified in several other proteins, some of which are known to be RNA-binding proteins. These include the archaebacterial ribosomal protein S3 [70] and the yeast meiosis-specific splicing regulator MER-1 [71,72] (see Fig.

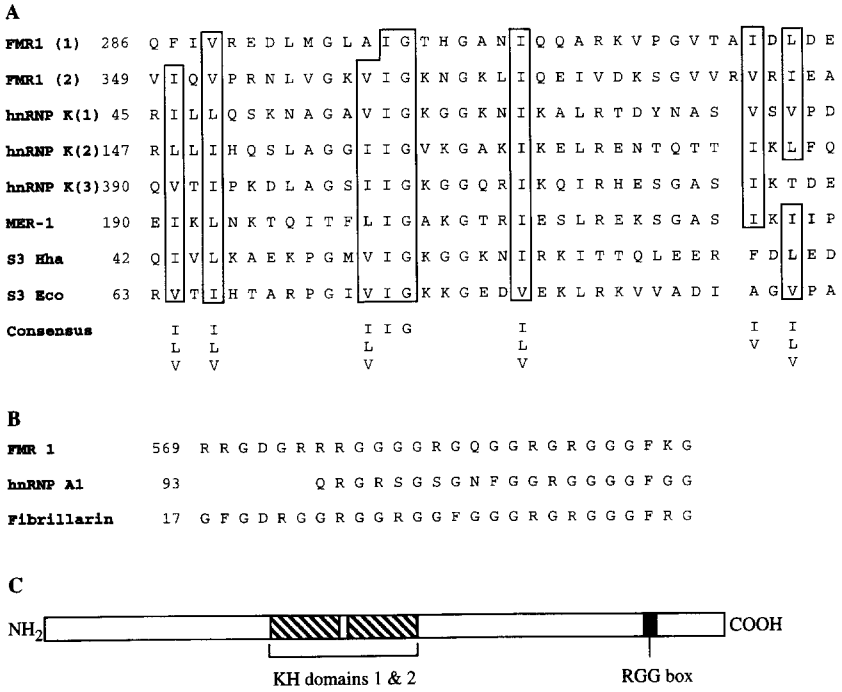

Fig. 5. Primary amino acid structure of the FMR1 protein and comparison to known RNA binding proteins. Letters refer to the single letter amino acid code. (A) KH domains in two regions of FMR1 protein showing similarity at the amino acid level to several known or putative RNA binding proteins. Highly conserved residues are boxed. (B) RGG box in FMR1 protein and similar domains in other RNA binding proteins. (C) Schematic representation of structural motifs within the FMR1 protein. The hatched boxes represent the KH domains and the filled box represents the RGG domain.

5A). An important piece of evidence that the KH domain is critical for the function of the FMR1 gene product is the finding of an individual with a very severe fragile X phenotype who does not carry the typical trinucleotide amplification, but instead has a single point mutation in one of the most highly conserved residues of the KH domain resulting in the conversion of Ile-367 to Asn [63].

A second domain, near the carboxyl end of the protein contains an arginine- and glycine-rich domain consisting of clusters of the tripeptide repeat termed the RGG box [73]. This motif has been demonstrated to have RNA binding activity and is present at the C-terminus of a number of nuclear RNA-binding proteins from diverse organisms. These include the hnRNP A1 protein, which is an abundant nucleoplasmic phosphoprotein [74], and fibrillarin [75] (see Fig. 5B).

Since both the KH domain and the RGG boxes are strong predictors of RNA binding activity, it has been speculated that the FMR1 protein (FMRP) may interact with other genes. The products of such genes could therefore vary in the absence of FMRP, contributing to the pleiotropic phenotype seen in the fragile X syndrome. FMRP has been transcribed and translated in vitro and the translation product shows strongest binding to the RNA homopolymer poly(G) [67]. Mutagenesis of conserved residues within either KH domain, or deletion of the RGG box all severely impair RNA binding [76]. All these studies, together with the phenotype of the individual with the point mutation, suggest that FMRP has an RNA binding function within the cell. These studies await the demonstration that FMRP is associated with RNA/protein complexes in vivo and it will be of particular interest to identify the cellular RNAs involved.

7. Expression studies

Studies on the expression of FMR1 show a high level of FMR1 expression in brain and testis [27,77]. Most other tissues show expression at a variable, but generally very low level. Interestingly, in the heart only small ($\sim$ 1.4 kb) mRNA species are seen, rather than the usual 4.4 kb transcript [78]. This is suggestive of tissue-specific differential splicing and, since mitral valve defects have been observed in fragile X syndrome, there may be physiological implications in the finding. Alternatively spliced forms of FMR1 have been demonstrated in several tissues using PCR of reverse transcribed cellular mRNAs [64]. However, similar studies have yet to be reported in heart.

Expression of FMR1 during development has been studied by the use of in situ hybridisation in the developing mouse [78] and in human fetal brain [79]. In the mouse embryo, fmr1 expression is detected throughout all tissues from the earliest stage studied (7 days gestation) until day 10, after which expression becomes more abundant in specific organs and regions, particularly in areas of the brain, liver and heart. The tissue specific variation becomes even more marked in the adult mouse, where high levels of expression are seen in the brain, testes, ovaries, thymus, oesophagus and spleen while there is no apparent expression in muscle, heart or aorta and only a low level of expression in most other tissues. The expression of FMR1 has been localised more precisely in the human fetal brain at two stages of development [79]. In 8- and 9-week-old fetuses, FMR1 mRNA is present in the proliferating and migrating cells of the nervous system, in the retina and in some non-nervous tissues. In 25-week-old fetuses, FMR1 mRNA is restricted to differentiated structures with the highest level being present in cholinergic neurons of the nucleus basilis magnocellularis and in the pyramidal neurons of the hippocampus.

The temporal and spatial pattern of expression of FMR1 in the embryo may explain the heterogeneous phenotype often seen in fragile X patients. This could arise from individual differences in when and where during fetal development the transition from premutation to full mutation, and subsequent methylation, occurs.

Antibodies raised to fusion proteins and synthetic peptides derived from FMR1 cDNA have been used to localise the protein at the subcellular level [80,81]. Immunohistochemical detection of FMR1 in the human central and peripheral nervous system localised it to neuron rich regions and not to the supporting cells. In the cerebellum, Purkinje cells lying between the granular and molecular layers were most intensely stained. Prominent staining was also seen in the granular layer, which consists mainly of small neurons. Staining in the brain cortex was confined also to neurons, particularly the cell bodies, with axons and dendrites being very poorly stained. In the testis, the protein was found exclusively in spermatogonia, with more mature cells being negative. No significant antibody labelling was observed in tissues of mesodermal origin, including the heart while all tissues of epithelial origin showed labelling, although at a lower level than that seen in the brain and testis. Interestingly, significant expression of the protein was particularly abundant in actively dividing cells. This situation contrasts with that seen in the brain, where only terminally differentiated neurons are labelled, but is in agreement with the mRNA in situ hybridisation data, which also suggest a high level of expression during cell division with a later specificity in regions of the brain. It is possible that FMR1 has more than one promoter responding to different regulatory signals, as is the case for other genes with dual or complex tissue specific expression patterns.

FMR1 protein transiently expressed in Cos-7 cells [80,81] is found almost exclusively in the cytoplasm. This is rather surprising, in view of its predicted role as an RNA binding protein, since proteins belonging to the hnRNP family have been observed almost exclusively in the nucleus [82–86]. However recent work has shown there can be shuttling of some members of the hnRNP family pro-

teins between the nucleus and cytoplasm [87]. This suggests that these proteins may also have functions within the cytoplasm. They may be involved in mRNA metabolism, such as the regulation of mRNA translation, stability and localisation. Most hnRNP proteins have signals which enable them to enter the nucleus [88], and the FMR1 protein contains a nuclear localisation signal near to its C-terminus which would suggest that it also has the ability to move between these cellular compartments.

8. Trinucleotide repeats and human disease

The finding of the trinucleotide expansion in fragile X syndrome signalled the identification of a new class of human mutations for which the term 'dynamic mutations' has been coined (for review see Sutherland and Richards [89]). The term is used to describe the unstable and continuously changing nature of the mutation. Myotonic dystrophy (DM) [90–95], Kennedy's disease (SBMA) [96], Huntington's disease (HD) [97], spinocerebellar ataxia (SCA1) [98] and dentatorubral-pallidoluysian atrophy (DRPLA) [99,100] have all been found to involve expansions of a CAG triplet repeat located in different positions within their genes. Another fragile site on the X chromosome (*FRAXE*) associated with mild mental retardation has a similar repeat structure to FMR1, with an expanded CGG repeat lying close to a CpG island which is hypermethylated in affected individuals [61].

The seven disorders characterised by trinucleotide repeats have many features in common and these are described in Table 1. All except one affect neuronal cells as their primary target. Fragile X syndrome results in mental retardation and is expressed specifically in neurons. In *FRAXE*, where the gene has not yet been identified, affected individuals exhibit mild mental retardation which manifests primarily as learning difficulties. The phenotypes of HD, SCA1, SBMA and DRPLA show the most striking similarities. Each is due to loss of particular subsets of neurons over time. In HD, cells of the striatum degenerate, resulting in chorea and eventual loss of cognitive functions. SCA1 shows loss of the neurons in the inferior olive in the cerebellum and brain stem, resulting in ataxia and motor weakness. In SBMA, dorsal root ganglia cells and motor neurons of the spinal and bulbar roots show degeneration, leading to muscular atrophy. DRPLA shows combined degeneration of the dentatorubral (cerebellar efferent) and pallidoluysian systems leading to progressive myoclonus, epilepsy, ataxia and dementia. DM differs somewhat from the other six disorders in that while there is neuronal involvement, muscle appears to be the primary tissue affected.

In common with fragile X syndrome, all of the disorders involving trinucleotide repeats show genetic anticipation. This manifests as variability in penetrance, a trend towards a more severe phenotype over generations or an earlier age of onset. Three of the mutations are X-linked (Fragile X syndrome, *FRAXE* and SBMA), while the others are autosomal (DM chromosome 19; HD chromosome 4; SCA1 chromosome 6; DRPLA chromosome 12). Apart from the presence of the trinucleotide repeat the genes show no similarities in their sequences.

In all the disorders where the gene has been identified, the trinucleotide repeat sequence is localised within the transcribed portion of the gene. This may suggest a functional basis for the trinucleotide repeats. In four of the disorders (HD, SCA1, SBMA and DRPLA) the repeats lie within the protein coding region and give rise to polyglutamine tracts. It may be significant that the repeats in both fragile X syndrome and DM lie in the untranslated region (UTR) of the gene (5′ and 3′ respectively). The expansions in the latter two disorders are very large, with increases of up to a hundred-fold, and as such would be unlikely to be tolerated within coding regions. This contrasts with HD, SCA1, SBMA and DRPLA where the repeat expansions are relatively small, and never exceed 100 repeats. The resulting protein products of these genes act in a manner consistent with a gain in function. This is most obvious in SBMA where the gene encodes an androgen receptor. Deletions within the gene cause loss of function resulting in testicular feminisation but not the muscular atrophy phenotype [94]. The mutation in the fragile X syndrome gives rise to a loss of function, due to abnormal hypermethylation. The situation in DM is far from clear. The product of the gene is a protein kinase (DM kinase). However the effect of the expansion in the 3′ UTR has not

Table 1
Disorders associated with trinucleotide repeat expansions

Disease	Repeat seq.	Range		Methylation	Location of repeat	Functional change
		normal	disease			
Fragile X syndrome	CGG	6–50	200–2000 +	yes	1st exon 5′ UTR	loss of function
FRAXE	GCC	6–25	200–850 +	yes	??	??
Myotonic dystrophy	CTG	5–37	50–2000	no	last exon 3′ UTR	? gain of function, ? mRNA stability
SBMA	CAG	12–34	40–62	no	1st exon coding	gain of function
Huntington's disease	CAG	11–36	42–100	no	1st exon coding	? gain of function
SCA1	CAG	19–36	43–81	no	? exon coding	? gain of function
DRPLA	CAG	8–25	54–68	no	? exon coding	? gain of function

been ascertained. The inheritance of the disorder is dominant, suggesting a gain-of-function mutation and it has been postulated that the increase in length of the 3′ UTR causes enhanced mRNA stability leading to overexpression or inappropriate expression of the gene [101,102].

9. Prospects

The involvement of trinucleotide repeats in neurological syndromes with unusual inheritance patterns has led to a search for candidate genes containing triplet repeats which may be responsible for other disorders [103–105]. In fact the DRPLA gene was isolated by searching the databank for cloned genes containing CAG repeats and using these to analyse patient DNA. The identification of such genes will have wide implications, both in terms of increasing knowledge about the functioning of neural cells within the brain and because it casts doubt on the long-held belief that DNA is a stable entity.

Trinucleotide repeat expansions have also provided explanations for a variety of phenomena which had been poorly understood. Genetic anticipation, incomplete penetrance and variable expression are all found where such expansions are the molecular basis for the disease. It is also becoming apparent that this type of mutation may be limited to humans, since the trinucleotide repeats so far examined in other species have not shown the same degree of polymorphism or tendency to expansion.

The identification three years ago of the gene defect involved in fragile X syndrome has had enormous impact on the diagnosis of the disease. The expensive and time consuming cytogenetic analysis of chromosome fragility has been largely replaced by detection of the CGG repeat by PCR or Southern analysis of DNA. Prenatal diagnosis can now be performed in early pregnancy and is offered to families of fragile X individuals. There have been proposals, notably in the United States, to extend this test to more widespread population screening on the basis of the high frequency of fragile X syndrome. However, there is debate regarding the ethical implications of such a programme. The disease is not life-threatening, but rather gives rise to individuals with mental and behavioural characteristics which do not lie within the accepted normal range. Furthermore, due to the incomplete penetrance displayed by the disorder the phenotypic consequences can range from very severe mental retardation to only slight behavioural problems. In addition, there is no prospect in the near future for effective treatment of the disorder either by conventional methods or by gene therapy, although some symptomatic treatment is being undertaken [106].

Despite intensive efforts, the precise molecular events which give rise to the fragile X phenotype are still unknown. Transgenic mice which lack the mouse homologue of FMR1 have been generated [107,108] but, although these animals have enlarged testes, they do not appear to show any abnormal brain pathology or overt behavioural or learning malfunctions. Much work is being focussed on the biochemistry of the FMR1 gene product and its possible role in RNA binding. This will be essential in elucidating the role of the gene product in intracellular events, and may give added impetus to the expanding field of RNA processing. The prospect of effective treatment for this disease may then become an attainable goal.

10. Note added in proof:

Since this review was written, expansion of a CAG triplet repeat in a gene on chromosome 14q32.1 giving rise to Machado-Joseph disease [109] and three further CGG triplet expansions associated with chromosomal fragility [110–112] have been described. This brings the number of examples of trinucleotide repeat amplifications to eleven.

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